World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Hematological indices and sensory quality of fermented soybean condiments

Iheukwumere CM [1,*] and Iheukwumere IH [2]

[1] *Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*
[2] *Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria.*

World Journal of Advanced Research and Reviews, 2022, 14(02), 435–442

Publication history: Received on 02 April 2022; revised on 13 May 2022; accepted on 16 May 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.14.2.0411

Abstract

Several studies have shown that most condiments consumed in Nigeria today are fortified with chemicals that alter the nature and affects the blood indices and the sensory parameters. Therefore the need to produced natural condiments from indigenous microbes in order to maintain the blood indices and the sensory attributes will be the ultimate success to be attained. The aim of this study was to evaluate the hematological indices and sensory quality of fermented soybean condiments produced from indigenous microbes. Soybean (*Glycine max*) sample was fermented with indigenous microorganisms isolated from 7 days old fermented soybean sample. This was oven-dried, pulverized and packaged in a cleaned sterile screw capped container. The hematological effects were determined using in vivo and instrumentation techniques. The sensory evaluation and acceptability of the condiments were carried out among validated participants and analyzed using 9-point hedonic scale. *Lactobacillus plantarum* strain ZS 2058 (L), *Bacillus subtilis* strain 168 (B) and *Saccharomyces cerevisiae* strain YJM555 (Y) were the indigenous microbes used singly and in consortium for the production of light to dark brown condiments with water activity ranging from 0.27 – 0.37 for the fermented soybean in the plate and 0.22 - 0.36 for the fermented soybean wrapped with *Thaumatococcus danielli* leaves (called *Uma* in Igbo and *Ewe* eran in Yoruba). The in vivo study showed that the condiment showed significant ($p<0.05$) increased in lymphocytes, slight increase in Red Blood Cells (RBCs) and little or no effect on other parameters, of the effects of BLY was most pronounced. The condiments fermented in sterile plastic plates had higher rating in odour, colour, taste and acceptability. Therefore condiments produced from fermented indigenous B, L, Y, BL and BLY positively affects the blood indices and had good sensory qualities and those fermented in plastic plates using BLY were most preferable and acceptable.

Keywords: Soybean; *Glycine max*; Soybean condiments; Fermentation; Sensory evaluation

1. Introduction

Studies have shown that soybean (*Glycine max* leguminosae) is one of the highly nutritional natural vegetable foods known to humans [1, 2]. It has been included in the world's top 5 healthiest foods in magazine 'Health (2006)' due to the nutritional and functional facts [3]. Also due to dozens of studies have shown that soy is good for the heart, the Food and Drug Administration even allows certain soy products to have a heart healthy claim on their labels. The origin and history of soybean dates back to 2838BC in China, and the emperor sheng-Nung of China named soybean as one of the five scared grain [4]. Soybean contains approximately 35%, protein, 31% carbohydrate, 17% fats, 5% mineral and 12% moisture [4]. The soybean protein contains acceptable amount of essential amino acids visa viz histidine, Isoleucine, leucine, lysine, phenylalanine, tryrosine, tryptophan and valine which is recommended for daily intake as a balanced diet [4]. In addition to essential nutrients, soybean products, especially fermented soybean products contain various functional components including peptides, isoflavonoids and saponin [3]. Soybean has been reported to improve cancer,

* Corresponding author: Iheukwumere CM
Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

improvement in bone mineral density and provide protection against bowel and kidney disease [2, 3, 5, 6]. These health benefits are caused by the presence of Isoflavone [2, 7], Saponins, protein and peptide in soybean [6-9].

Soybean as a food have many different formulations such as soymilk, soyflour, soy oil, feed for livestock and poultry, soy concentrate, protein isolates, soy yoghurt, tofu and fermented foods such as Tempeh, soy sauce, *misco,* Natto and *sufu* [1]. Although soy bean has high protein content, minerals, vitamins and bio-actives, it has little direct use because of high oil content, poor digestibility, green beany taste, long cooking time and persistent bitterness. Fermentation is a proven method used to improve flavor, texture and nutritional quality of soybean. Besides bringing physicochemical and sensory quality changes, fermentation also helps towards the preservation of food due to release of metabolites that discourage the growth of pathogenic bacteria in foods [1]. These fermented soybean are used as condiments to flavor foods such as stir-fries, stew, and soups [3].

Fermentation involves a range of microorganisms such as lactic acid bacteria, acetic acid bacteria, yeasts, mould and *Bacillus subtilis*. Some examples of soybased foods includes: Japanese *Hikiwari, Natto, itohiki-Nattto:* Thai *Thua –Nao;* Chinese *Shui-Toushi:* Nigeria Daddawa or Dawadawa; and other legume oil seed based fermented products from different regions of Africa [1]. These traditionally fermented soy foods are littered with several other microbial species such as *Enterococcus faecium, Geotrichum candidum, Aspergillus oryzae* etc but *Bacillus* remains the sole organism carrying out the fermentation. One of the most reported health benefits of *B. subtilis* fermentation is almost complete removal of indigestible oligosaccharides (stachyose) which are responsible for indigestion and flatulence in humans and manogastric animals [10]. They have also shown to reduce the activity of anti-nutrients that hinders availability of proteins and phytochemicals present in soybeans, also they completely remove the beany odour of raw soybeans and increases sensory quality of the product [1, 11]. Kyriakis *et al.* [12] suggested that probiotics from strains of lactic acid bacteria and *Bacillus* spp could be used as alternative feed additives to piglets so as to replace antibiotic and antimicrobial compounds in their feeds which may contribute to antibiotic resistance in human. Kalari *et al.* [13] also claimed that *Rhizopus* fermented soybeans (Tempeh) reduces the duration of diarrhea when added to the diet of malnourished children. Kiers *et al.* [14] showed that while fermented soybeans inhibits adhension of ETEC K88 in *Rhizopus* fermented soybeans, *Bacillus* which acts as probiotics allows *Bacillus* fermented products to inhibit the proliferation of pathogens in gut. Wei *et al.,* [15] reported that isoflavone which acts like oestrogen is able to reduce risks related to cardiovascular diseases, lower rate of prostrate, breast and colon cancers and improve health benefits. Similarly, Kon *et al.* [16] linked consumption of fermented soybeans with reduction of diabetes type -2 which improved glucose control and insulin resistance.

Unfortunately, most of the foods consumed today are laden with sweeteners, artificial flavor, coloring agents and chemicals that alter texture of the foods, but the trouble is not just what has been added, but what has been taken away. These foods are often stripped of nutrients designed by nature to protect your heart, such as soluble fiber, antioxidants and good fats. Some of the foods are associated with neurological disorders, allergic reactions, hyperactivity, fertility cases, attention deficit disorderly especially in children and other cases. Recently, bioactive compounds associated with fermented products are considered as an important strategy to increase soluble fibers, proteins and reduce fat contents of foods, and to boost body immunity. Isoflavones and phenolics are the main bioactive compounds that improve growth performance, antioxidant activity and immune function [2, 17-19]. Several condiments have been produced from fermented soybean in developed countries but there is still a controversial thought in the sensory quality and hematological indices of these condiments, therefore this present study is aimed at evaluating the hematological indices and sensory quality of fermented soybean condiments.

2. Material and methods

2.1. Sample Collection

This was carried out using the modified method of Suleiman and Omafe [20]. Soybean seeds were collected randomly from different shops and open markets in Eke Awka, Awka South LGA. Anambra State. Sampling was performed manually from different bags and basins, such that soybean seeds were collected from different parts of the bags and basins. The samples were aseptically pooled and mixed properly to form a bowl and placed in sterile nylon bag, the soybean seeds were properly labeled and taken to the laboratory for analysis.

The albino Wistar rats used in this study were purchased at the animal house, Zoology Department, University of Nigeria, Nsukka (UNN). The rats were transported to the animal house at the Department of Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University (NAU), Awka. The rats were critically examined for their weights and experimented for their suitability for the study. The rats were selected and grouped based on their weights and experimented design.

In vivo Study: A total of 52 albino Wistar rats were used for this study. The rats were grouped into 13 groups. Each group contained 4 rats. The rats were orally administered 1.0 g/ kg (tenfold of normal administration) of the prepared condiments except for the last group that was given ordinary distilled water as normal control. The rats in each group were monitored for 21 days.

2.2. Transportation

A sterile polythene bag containing ice blocks placed inside a cooler was used for the transportation of the sample. The temperature of the cooler was carefully checked and adjusted to 28^0C -30^0C in order to prevent or reduce microbial shock by reducing the quality of the ice inside the cooler. The samples were aseptically arranged inside the cooler without direct contact with the ice bag. The cooler was covered properly with packing tape to prevent accidental opening of the cooler. The cooler was taken to the laboratory safely for the analysis.

2.3. Preparation and Local Fermentation of the Soybean

Two hundred and fifty grams of cleaned soybean seeds were weighed using an analytical weighing balance and steeped in 500ml bucket of water overnight, after which the seedcoat were removed by rubbing between the palms and then the chaff were removed using sieve. The soybean seed were then thoroughly washed and placed inside cleaned *Thaumatococcus danielli* leaves (called "uma" in Igbo and "ewe eran" in Yoruba) and wrapped properly and then kept inside 500ml bucket that was well covered with the lid for fermentation to take place for 7 days at room temperature.

2.4. Processing of the Fermented Soybean

After the fermentation the fermented soybean were prepared for culturing and the diluents used was peptone (BIOTECH) water which was prepared according to the manufacturers instruction, then was sterilized by autoclaving at 121^0C for 15 min at 15 psi. Ten grams of the fermented soybean was aseptically weighed using analytical weighing balance into a 200 ml beaker (G.G) and little amount of the diluent was added and homogenized and then make upto 100 ml, part of these preparations was transferred into 100 ml beaker (G.G) and boiled for 10-15 min using a pressure pot.

2.5. Isolation of the Test Sample

The media used for this isolation includes Sabourand dextrose agar (SDA), de Man Rogosa and Sharpe broth (MRS) and Nutrient agar (BIOTECH). A 0.1ml of the preparation/inoculum collected using a sterile pipette and aseptically plated onto solidified Sabourand dextrose agar plate (90 mm x 15 mm) which was prepared according to the manufacturers instruction and the procedures described in Cheesbrough [21] supplemented with chloramphenicol (0.05%) and spread using a spreading rod, 0.1 ml of the boiled preparation/inoculums was collected and plated unto solidified nutrient agar plate also 1 ml of the inoculums was collected using sterile pipette and aseptically inoculated into sterile 100 ml conical flask (Glassco) containing MRS broth (Oxoid) which was prepared according to the manufacturers instruction and the conical flask were incubated in a microaerophic environment (containing candle used to evacuate all traces of oxygen thereby creating an environment having only carbon iv oxide). The incubation was done for 24 – 72 h at (30±2 ^{0}C). The SDA and NA were incubated in an inverted position for 24 h at 35±2 ^{0}C (for NA) and 30±2 ^{0}C (for SDA) in an incubator (STXB128). The isolates were sub cultured and characterized appropriately.

2.6. Preparation of Soybean Condiments

2.6.1. Processing of soybean for fermentation

This was carried out using the modified method of Farinde *et al.* [22]. One kilogram of soybean were carefully picked and weighed using analytical weighing balance and steeped in 200ml bucket of water overnight for fermentation to take place, after the soybean were dehaulled by rubbing between the hands to remove seed coat, after the chaff/seed coat were properly removed using a clean sieve, the soybean was then properly washed and placed inside a beaker and then autoclaved at 121^0C for 15 min at 15psi.

2.6.2. Fermentation Process

This was carried out using the modified method of Hu *et al.* [11] and Chukeatirote *et al*. [23]. After autoclaving the soybean, a 100g of soybean was weighed using analytical weighing balance and placed inside 6 different *Thaumatococcus danielli* leaves (called "uma" in Igbo and "ewe eran" in Yoruba) which was properly sterilized using electric oven at 180 ^{0}C for 2 h, each of the leaves containing the soybean were inoculated with the fermenters prepared and diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.6mL of 1% $BaCl_2$. $2H_2O$ and 99.4 mL of 1% Conc. H_2SO_4, 10 ml of suspension *Bacillus* was added and labeled as "B" , 10ml of suspension of

Lactobacillus was added and labeled as "L", 10 ml of suspension of yeast was added and labeled as "Y" , consortium of suspensions 5 ml of Bacillus and 5 ml of Lactobacillus was added and labeled as "BL", consortium of suspensions of 3ml of Bacillus, 3 ml of Lactobacillus and 4 ml of yeast were added and labeled as "BLY" consecutively and one of the leaves containing only soybean was set aside as the control. These leaves were carefully wrapped. This same method was repeated using sterile plates. The wrapped leaves and the plates containing the soybean were kept at room temperature for fermentation to take place for 7days.

2.6.3. Storage and packaging

After fermentation, the fermented samples were aseptically dried using an electric oven at 80^0C for 7days**.** After drying water activity of the fermented samples was determined, after which it was grinded into powder and stored in a sterile screw capped container for subsequent analysis.

2.7. Hematological Indices of the Blood Samples from the Experimented Rats

2.7.1. Hematological Indices

The blood samples collected from the scarified rats were examined using Automated Hematology Analyzer (MIN DRAY BC – 360), and the variations in the red blood cells (RBCs), lymphocytes, monocytes, neutrophils, eosinophils and basophils were assessed and recorded as described in the work published by Agiang *et al.* [24].

2.8. Sensory Evaluation and Acceptability of the Condiments

2.8.1. Sensory Evaluation

In house consumer-oriented test was conducted to determine product acceptability using scoring test with the aid of 9-points hedonic scale with little modification in the studies published by Adedokun *et al*. [25] and Enidiok *et al*. [26]. The sensory characteristics of the condiments such as colour, odour, taste and general acceptability were examined by the team of twenty (20) validated panelists which was drawn from microbiology students of Nnamdi Azikiwe University, Awka. The panelists were validated in such a way that they were able to detect little perceptible changes in the sensory attributes mentioned. Each panelist was asked to score each coded sample based on a nine point hedonic scale (like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely).

2.8.2. Data Analysis

The data obtained in this study were presented in tables and figures. Their percentages were also calculated. The sample means and standard deviations of some of the analytical data were also calculated. The significance of the prevalence of the isolates in the studied samples was determined at 95% using one way analysis of variance (ANOVA). Pairwise comparison was carried out using student "t" test.

3. Results

The study revealed that the prepared condiments had pronounced activity on some hematological parameters of the experimented rats (table 1). There were significant ($P<0.05$) increase in the total white blood cell (WBC) counts of the blood samples of those rats fed with condiments prepared with BP, BL, YP, YL, and "okpeyi" . The study also revealed that the condiments had significant ($P<0.05$) effect on the Lymphocytes of the blood samples drawn from the experimented rats. The Lymphocytes was significantly ($P<0.05$) higher among the rats fed with condiments prepared with BLP, BLL and BLYP and BLYL of which the increased was most from pronounced for BLYL. Those rats fed with condiments prepared with BP, BL, LP, LL, YP, YL and okpeyi showed non - significant ($P>0.05$) increase in blood Lymphocytes. There was none - significant ($P > 0.05$) decreased in those rats fed with condiments prepared with CP, CL and maggi when compared with the control group. There were significant ($P<0.05$) decrease in blood neutrophils from blood samples drawn from rats fed with condiments prepared with BLL, BLYP and BLYL.

Also the study revealed that the blood samples drawn from the experimented rats showed no significant changes in monocytes and basophils but significant changes in eosinophil were seen on the blood samples drawn from those rat fed with condiments prepared with BL, LP, LL, YP, BLP, BLL, BLYP, and BLYL. There was no significant difference ($P > 0.05$) between the value of the RBC of the control and that from the experimented rats.

The sensory evaluation/parameter of the prepared condiments is shown in Table 2. The study revealed that the colour, taste, odour and the general acceptability of the prepared condiments were within dislike slightly and like very much.

The color of the condiments prepared from BP, LL, YP, BLP and BLYP (plates) were within like slightly to like moderately where's those condiments prepared from BL, LL, YL, BLL and BLYL (leaves) recorded dislike moderately to neither like now dislike colour. Also the odour of those condiments prepared in plates (BP, LP, YP, BLP and BLYP) ranged from neither like nor dislike to like slightly whereas those prepared in leaves (BL, YL, LL, BLL and BLYL) ranged from dislike slightly to neither like nor dislike. Similar trend were seen in the taste of the condiments the general acceptability of the condiments prepared in a plate ranged from like moderately to like very much whereas those prepared in leaves red exhibited similar hedonic point. The study revealed that those condiments prepared in the plates showed better colour, taste, odour and more acceptable than those condiments prepared in leaves.

Table 1 Hematological indices of the blood samples drawn from rats fed with prepared condiments

Sample	WBC (c109L	Neu (%)	Lym (%)	Mon (%)	EOS (%)	Bas (%)	Nev (x109L)	Lym (x109L)	Mon (x109l)	Eos (x109L)	Bas (x109L)	RBC (x1012L)	PLT (x109L)
Bp	30.78	34.6	58.8	0.0	6.5	0.1	10.65	18.10	0.00	2.00	0.03	5.28	944
BL	18.62	37.9	60.2	0.4	1.0	0.5	7.06	11.21	0.07	0.19	0.09	6.40	745
LP	10.25	33.6	64.9	0.7	0.2	0.6	3.44	6.66	0.07	0.02	0.06	5.69	432
LL	8.56	34.1	64.3	0.6	0.1	0.0	2.92	5.51	0.05	0.08	0.00	5.47	785
YP	20.36	40.8	56.8	0.5	1.2	0.7	8.31	11.57	0.10	0.24	0.14	5.74	1000
YL	33.28	34.8	57.0	0.1	7.8	0.3	11.59	18.97	0.03	2.60	0.09	5.43	280
BLP	8.65	32.8	66.1	0.3	0.5	0.3	2.84	5.72	0.02	0.05	0.02	4.53	63
BLL	12.77	28.2	67.3	0.2	3.8	0.5	3.61	8.60	0.02	0.48	0.06	5.52	662
BLYP	15.75	27.2	71.1	0.3	0.8	0.5	4.30	11.20	0.04	0.13	0.08	5.12	471
BLYL	3.55	7.2	91.5	1.0	0.3	0.0	0.26	3.25	0.03	0.01	0.00	5.66	49
Maggi	11.25	41.4	43.9	0.6	13.4	0.7	4.67	4.96	0.06	1.52	0.07	6.12	502
Opei	20.79	24.3	55.2	0.1	20.1	0.3	5.06	11.48	0.02	4.17	0.06	4.17	566
Cp	15.02	44.1	46.6	0.5	8.2	0.6	6.63	7.00	0.07	1.23	0.09	6.02	715
CL	11.43	36.1	46.7	0.6	15.8	0.8	4.13	5.34	0.06	1.81	0.09	6.61	653
C	12.74	40.6	49.3	0.2	9.7	0.2	5.18	6.29	0.02	1.23	0.02	7.27	805

Table 2 Sensory parameters of the prepared condiments

Sample	Color	Taste	Odor	General Acceptability
Bp	0.61±0.02	0.52±0.00	0.54±0.01	0.74±0.07
BL	0.43±0.04	0.51±0.01	0.44±0.00	0.71±0.03
LP	0.63±0.01	0.53±0.00	0.55±0.01	0.78±0.05
LL	0.44±0.01	0.52±0.01	0.46±0.01	0.73±.03
YP	0.60±0.03	0.49±0.00	0.51±0.00	0.74±0.01
YL	0.41±0.02	0.47±0.00	0.48±0.00	0.70±0.02
BLP	0.58±0.01	0.51±0.00	0.57±0.01	0.77±0.03
BLL	0.38±0.03	0.50±0.00	0.51±0.01	0.73±0.04
BLYP	0.62±0.02	0.52±0.01	0.59±0.02	0.79±0.03
BLYL	0.40±0.01	0.50±0.01	0.51±0.01	0.74±0.06

4. Discussion

Variations in the values of immune cells/blood cells observed in the present study could be attributed to the variation in the ability of the condiments to augment the hematopoietic processes in the cells of the experimented rats. Sensory quality is an important indicator of the overall quality and health safety of food products. The sensory characteristics of the prepared condiments in the present study conform to the good quality and safety of the condiments. Similar findings and ideas were reported by Adedokun *et al.* [25] and Enidiok *et al*. [26]. The variation in the hematological parameters of the albino Wistar rats fed with the condiments agrees with the findings of Jacob Filho *et al*. [27]. The increase in white blood cells observed in the present study agrees with the findings of Bamidele *et al.* [28] but disagrees with the findings of Femi – Oloye *et al*. [29] who studied the effects of commonly used food additives on hematological parameters of Wistar rats. The increase in red blood cells (RBCs) observed in this study agrees with the findings of Enidiok *et al.* [26] but disagrees with the findings of Bamidele *et al*. [28]. The increase in WBCs and lymphocytes found among the rats fed with the condiments could be attributed to the phytochemical constituents of the soybean used in the production of the condiments. Scientists reported that glycosides possess anti – inflammatory properties which increase the level of WBCs.

5. Conclusion

This study has shown that condiment produced from fermented soybean using indigenous *Bacillus subtilis* strain 168 (B), *Lactobacillus plantarum* strain ZS2058 (L) and *Saccharomyces cerevisiae* strain YJM555 (Y) were safe, were able to argument the hematopoietic processes in the experimented rats and had good sensory quality, of which those condiments produced using consortium of BLY were most preferable and acceptable.

Compliance with ethical standards

Acknowledgments

We acknowledge everyone who provided one help or the other during this study. Our appreciation also goes to our students who helped during the experimental phase.

Disclosure of conflict of interest

Authors declare no conflict of interest.

Statement of ethical approval

Approval for the use of laboratory animals was obtained from Chukwuemeka Odumegwu Ojukwu University laboratory care and animal committee.

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